RESEARCH PROPOSAL

ROLE OF POLYMORPHISMS IN DOUBLE STRAND BREAK REPAIR GENES AND HEAD AND NECK CANCER INCIDENCES

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INTRODUCTION

Head and neck cancers (HNCs) influence mainly the upper aero digestive tract and are one of the most common cancers worldwide. Squamous cell carcinoma of head and neck (HNSCC) is the fifth most common type of cancer worldwide and second in the developing world (Parkin *et al.,* 2002, Marcin and YeoH, 2009). In the Indian subcontinent, HNC is the most common malignancy, accounting up to 40- 50% of all malignant cancers (Ferlay *et al.,* 2001). HNCs are a far reaching problem in India constituting approximately one-third of all cancer cases in contrast to 4–5% in the developed world. The universal factors responsible for HNCs worldwide are smoked tobacco and alcohol and the etiological constituents liable for it in the Asian and Caucasian population are smokeless tobacco, betel nut, and Epstein–Barr virus (Wang *et al*., 2015).

Inherited genetic variation plays a critical but largely uncharacterized role in human differentiation. Variations in the DNA sequences of humans can affect diseases and response to pathogens, chemicals, drugs, vaccines, and other agents. Genetic variation in human genome is an emerging resource for studying cancer. Molecular epidemiological studies have provided ample evidence that an individual's susceptibility to precancerous lesions and cancer is modulated by both genetic and environmental factors.

Although many people are exposed to these risk factors, only a fraction of exposed individuals develop cancer, suggesting an individual susceptibility to exposure-related carcinogenesis. Due to significant alteration in environmental carcinogens a defective DNA damage response is induced, which ahead's toward apoptosis or genetic instability and un-regulated (proliferative) cell growth.

The risk of Head and Neck cancer is boosting more due to transformed DNA repair extent. With increase in cases of HNC, treatment options are also increasing and amongst them one is radiation therapy which significantly boosts up acute as well as late toxicities. There are various treatments like radiotherapy techniques, biologically effective dose, use of concurrent chemotherapy (dose and type of chemotherapy) and energy of radiation beams etc. which are diagnosed as prognostic factors helps in enhancement of acute radiation distress (Marcu L. *et al*., 2009). But these treatments are unable to explain the inter-individual variability present in patients in reference to the beginning and development of acute toxicity.

As we know various DNA repair pathways are present, which provide apparent but overlapping conservation across mutagenic exposures. Nucleotide excision repair (NER), base excision repair (BER), and double strand break (DSB), are the three essential DNA repair pathways which helps in maintenance of genomic integrity. There are many DNA repair genes such as OGG1, XRCC1, XRCC2 and XRCC3 present in various individuals suffering from HNC. The importance of different single nucleotide polymorphisms (SNPs) in DNA repair genes as a prognostic marker of acute radiation toxicity has been discussed by various researchers (Andreassen , 2010; Wiebalk *et al*., 2007; Chang-Claude *et al*., 2005; Mangoni *et al*., 2011; Azria *et al*.,2008, Alsner *et al*., 2008).

This study mainly focuses on XRCC1 and XRCC3 which are present in BER pathway and plays an extensive role in repairing of DNA injury induced by ionizing radiation. The first gene which impacts the responsiveness of cells towards ionizing radiation in mammals is X-ray repair cross-complementing gene 1 (XRCC1). It encrypts the proteins which interact with poly-ADP-ribose polymerase (PARP), DNA polymerase-beta (POLB), DNA ligase III (LIG3). In XRCC1 gene, three prevalent polymorphisms are Arg194Trp, Arg280His and Arg399Gln which undergo amino acids substitution. XRCC1 Arg399Gln consists of modified alleles which have transformed enzyme functions, superior ascent towards DNA addict and a vigorous corporation with cancer (Yu *et al.,* 2004; Choudhury *et al.,* 2014).

Another family of genes liable for reformation of DNA double strand breaks provoked by normal metabolic procedure and/or exposure to ionizing radiation is X-ray repair cross-complementing group 3 (XRCC3). It is present in the 14q32.3 chromosome region. In homologous recombination repair system, the function of XRCC3 is not completely understandable, in spite of this; it shows coherence with Rad51, which mobilizes DNA strand exchange in homologous recombination, and XRCC3-deficient cell lines present compressed homologous recombination repair.

The universal polymorphisms in exon 7 of the XRCC3 gene sequence towards amino acid substitution at codon 241 (Thr241Met) alter the enzyme functions and its interaction with other proteins involved in DNA damage and repair. Preceding analysis recommend that single nucleotide polymorphisms (SNP) in XRCC1 and XRCC3 perhaps correlated with head and neck cancer risk, but the findings have been unpredictable.

REVIEW OF LITERATURE

As we all know genetic vision plays a crucial role in regulation of each gene which leads to development of cancer. Today's era mainly focuses on polymorphisms in DNA repair genes as a dominant component of susceptibility because DNA-repair activities are important for the stability of the genome and the prevention of cancer. SNPs were detected in every human DNA repair gene through various methods, and were used to regulate the levels of DNA damage, individual DNA repair capacity and cancer risk (Goode *et al.,* 2002). Double-strand breaks present in DNA leads to cell death or in genetic material breakage and unrepaired leads to genetic instability and even results in cancer development. So, we can focus on DSBr (Double strand break repair) gene in tumor cells as an arm for handling emerging problems.

The DNA repair capacity is reducing now days according to various researches which leads in genetic polymorphisms in different DNA repair genes, that are related to rising possibility and uncertainty towards different types of carcinoma in human beings (Shen *et al*., 2000; David-Beabes *et al*., 2001; Ratnasinghe *et al*., 2001; Hao *et al*., 2004; Zhi *et al*., 2004; Zhang *et al*., 2005). The polymorphism in DNA repair genes has been extensively investigated for its associations with cancer risk and the results were conflicting in different types of cancer or different populations (Hu *et al*., 2005; Zeng *et al*., 2009; Wei *et al*., 2010; Saadat 2010). Flores-Obando *et al*., 2010 not able to recognize significant association between alcohol, human papillomavirus, smoking, ethnicity and DNA repair gene polymorphisms in XRCC1 and oral cancer.

XRCC1

X-ray repair cross complementing gene is involved in DNA single strand break repair by mediating the assembly of DNA break repair protein complexes. During the process of DNA repair the XRCC1 gene negatively regulates the ADP ribose levels by modulating ADP ribosytransferase PARP1 activity. The molecular function of XRCC1 gene are damaged DNA binding, DNA ligase activity, enzyme binding, oxidized DNA binding, protein binding and 3' overhang single strand DNA endo deoxyribonuclease activity.

The cellular components of XRCC1 gene are nucleoplasm, nuclear chromosome telomeric region, nuclear, chromatin, and nucleolus and cell nucleus. The over expression of this gene leads to carcinoma because it is intermediated by MMEJ (Microhomology mediated end joining pathway responsible for repairing ds breaks in DNA) repair which is directly mutagenic.

It plays crucial role both in Base excision repair and single strand break repair and even responsible for removal of oxidatively damaged DNA which is caused by exposures to ionizing radiation or alkylating agents.

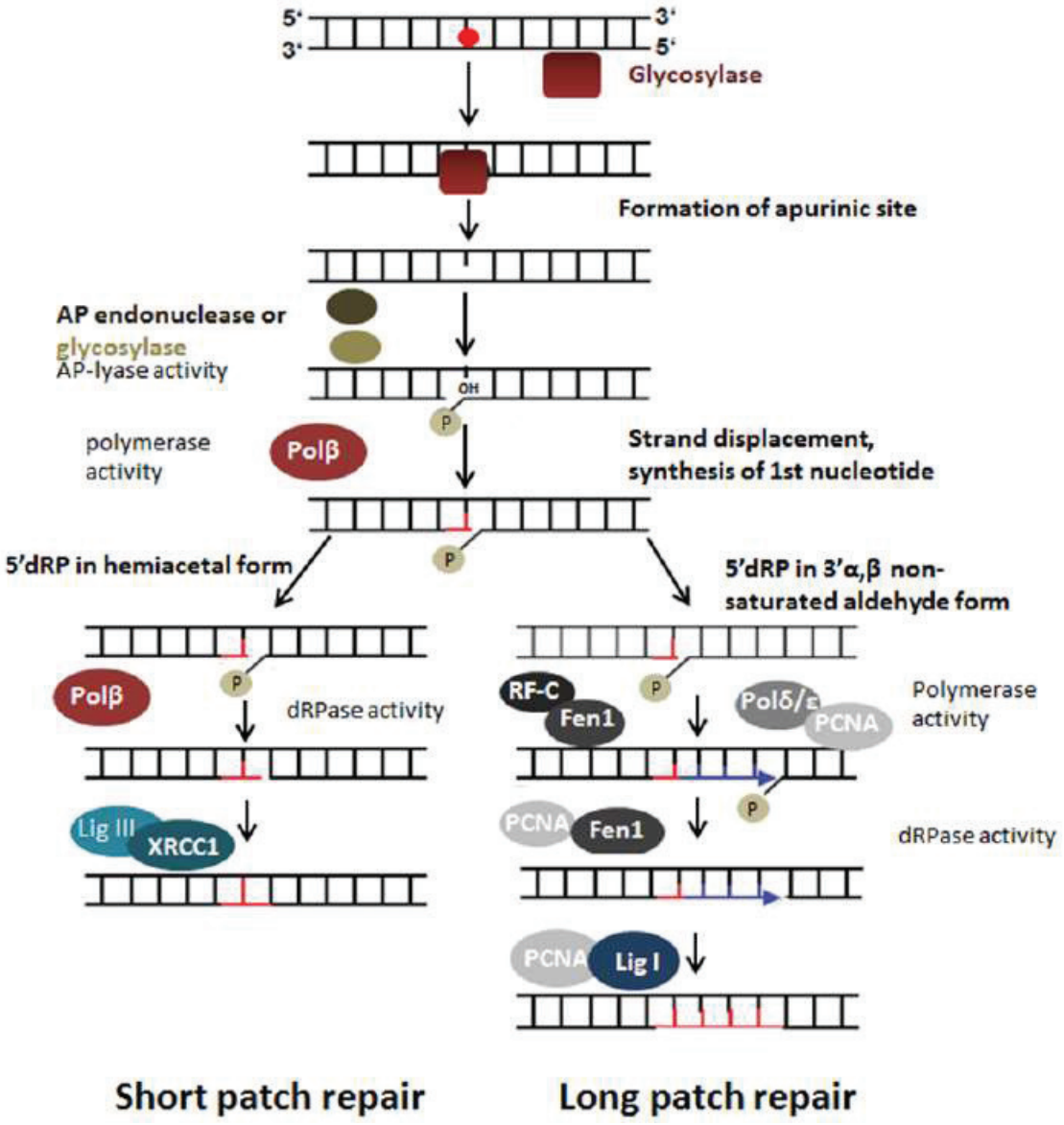
This gene also interacts with DNA ligase III polymerase beta and poly (ADP- ribose) polymerase for involving itself in the base excision repair pathway (BER pathway). It is also significant during DNA processing when myogenesis and recombination in germ cells occurring. It also activates the repair activity performed by hoGG1 on DNA containing OxoG, which is similar to stimulation by APE1.

BER PATHWAY (Base excision repair pathway)

It is a pathway which involves removal and replacement of damaged or nonintegrated bases present inside the DNA strand that might cause mutation if left unrepaired. The damaged base is modified and excised to create a single-stranded DNA break that is repaired to regenerate an intact helix. Base excision repair is an important and uppermost pathway responsible for correcting non-helix distorting lesions which are introduced by ionizing radiation or cellular metabolic events. Other pathways are also present for direct reversal of certain types of damage (e.g., photolyase and methyl transferase), removal of inter-strand crosslinks and repair of DNA strand breaks and correction of mismatched bases.

Excision repair involves removal of a damaged nucleotide by dual incisions bracketing the lesion; this is accomplished by a multisubunit enzyme referred to as the excision nuclease or exonuclease. The basic mechanism of excision repair involves: (1) damage recognition; (2) subunit assembly; (3) dual incisions that result in excision of the damage-containing oligomer; (4) resynthesizes to fill in the gap; and (5) ligation to regenerate an intact molecule.

This pathway is normally started by lesion-specific DNA glycosylases and completed by either of the two sub-pathways: short-patch BER pathway in which one nucleotide is changed and long-patch BER pathway in which 2-13 nucleotides are replaced. Both pathways of BER depend on the protein complexes formation which assemble at the site of the DNA lesion and improves repair in a defined fashion. This process of complex formation appears to provide an increase in specificity and efficiency to the BER pathway, thereby facilitating the maintenance of genome integrity by preventing the accumulation of highly toxic repair intermediates.



Schematic representation of Base Excision repair pathway which includes two sub pathways short patch repair and long patch repair (Leyns *et. al.*, 2012)

XRCC3

XRCC3 (X-Ray Repair Cross Complementing 3) is a Protein Coding gene. The diseases which occur due to some defect in XRCC3 are as follows Cutaneous Malignant 6, Breast Cancer and Melanoma. The important pathways which are regulated with the help of XRCC3 gene are as follows Integrated Breast Cancer Pathway and Homologous DNA Pairing and Strand Exchange. Gene Ontology (GO) annotations related to this gene include DNA-dependent ATPase activity and crossover junction endo deoxyribonuclease activity.

This gene is structurally associated with RecA/Rad51-related protein family which participates in homologous recombination repair of DNA double strand breaks and cross links and even maintain chromosome stability. It is required for the assembly and stability of Rad51.The gene functionally accompanies Chinese hamster irs1SF (a repair-deficient mutant) which presents hypersensitivity towards various DNA-damaging agents and is chromosomally unstable.

The cell which does not consist of Rad51 foci are damaged after radiation and even goes through genetic instability and even increases their sensitivity towards DNA damaging agents such as ultraviolet rays. This gene contains unique microsatellite polymorphism associated with cancer in patients of varying radio sensitivity. Alternatively, spliced transcript variants encoding the same protein have been identified.

XRCC3 is polymorphic for a C-T substitution at exon 7position which results in an amino acid change. It is involved in homologous recombination repair pathway of double stranded DNA, thought to repair chromosomal fragmentation, translocations and deletions. Anon-synonymous mutation is present in exon 7 (Thr241Met) of XRCC3 was found to be associated with higher levels of DNA adducts and reduced repair capacity. This mutation is also associated with p53 mutations and increased cancer susceptibility.

This gene plays efficient role in homologous recombination repair (HRR) pathway of double-stranded DNA, and also helps in chromosomal fragmentation, translocations and deletions. When some damage occurs in DNA, CX3 acts downstream of RAD51 recruitment and helps this complex in binding prior to the four duplex arms intersection presents inside Holiday junction (HJ) and even to the replication forks junctions.

This gene also shows its efficacy in HJ resolution and also in formation of HR intermediates in last of DNA repair process and its role can be associated with CX3 complex and also shows the involvement of GEN1 in mitotic cell cycle progression. The important function of this gene is to regulate mitochondrial DNA copy number even in presence of oxidative stress in front of RAD51 and RAD51C.

HRR PATHWAY (Homologous recombination repair pathway)

It is a type of genetic recombination in which nucleotide sequences are exchanged between two similar or identical molecules of DNA. This pathway is widely used by cells for repairing harmful breaks properly which are present on both strands of DNA and commonly known as double-strand breaks.

This mechanism results in a loss of heterozygosity or genetic rearrangements. These genetic alterations may play initial role in carcinogenesis, but they are more likely to be involved in secondary and subsequent steps of carcinogenesis by which recessive oncogenic mutations are revealed.

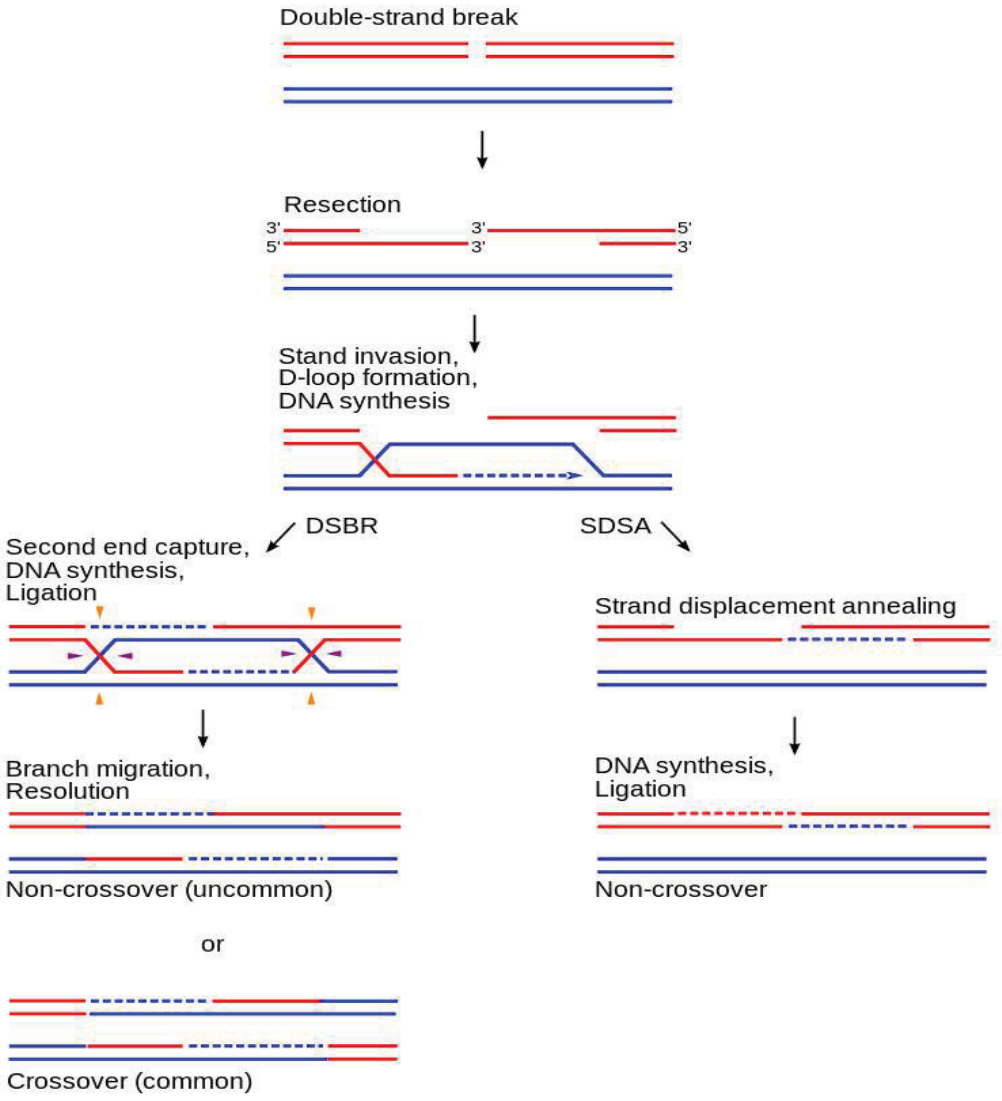


Table 1: Studies conducted on XRCC1 and XRCC3 genes (International)

S.No.	Author and year	Title of paper	Outcomes of paper
1	Bashir *et al*., 2018	Haplotype analysis of *XRCC1* gene polymorphisms and the risk of thyroid carcinoma	XRCC1 Arg280His and Arg194Trp were associated with thyroid cancer in Pakistani population. These genetic markers may provide an insight into the disease pathogenesis and help open novel therapeutic strategies for thyroid cancer.
2	Avci1 *et al*., 2017	A Strong Relationship between Oral Squamous Cell Carcinoma and DNA Repair Genes	The XRCC1 Arg399Gln Gln/Gln genotype and Gln allele were risk factors for OSCC. Also, Arg/Arg genotype and Arg allele had protective effects against OSCC.
3	Chen *et al*., 2017	Association between XRCC1 single-nucleotide polymorphism and acute radiation reaction in patients with nasopharyngeal carcinoma A cohort study	This study confirmed an association between XRCC1 codon399 genotype and the risk of acute radiation dermatitis in locally advanced NPC patients. However, due to the small sample size, analysis of additional genes in combination with various biomarkers was not possible. In the future, multicenter studies with a larger sample size should be performed to explore the value of genetic factors on predicting radio sensitivity. This further provides more powerful theory to support individualized radiotherapy based on molecular markers.
4	Halkova *et al.,* 2016	Polymorphisms in selected DNA repair genes and cell cycle regulating genes involved in the risk of papillary thyroid carcinoma	It appears that the development of PTC could be caused by different genetic events depending on gender and that influence of some SNPs of XRCC1 in low penetrance genes could be more pronounced in men than in women and vice versa. It seems that individual genetic background could modulate the onset of cancer and clinical behavior of tumor.
5	Yang *et al*., 2015	A Systematic Gene–Gene and Gene–Environment	XRCC1 Arg194Trp of DNA repair genes were significantly

		Interaction Analysis of DNA Repair Genes XRCC1, XRCC2, XRCC3, XRCC4, and Oral Cancer Risk	associated with oral cancer susceptibility and this risk may be enhanced with exposure to certain environmental factors.
6	Stur *et al*., 2015	Prognostic significance of head and neck squamous cell carcinoma repair gene polymorphism	The XRCC1 399Gln, XPC 939Gln, XRCC3 241Met, and RAD51 -98G>C alleles may be used as genetic markers for poor prognosis in oral, pharyngeal and laryngeal tumors.
7	Santos Pereira *et al*., 2015	Association of the XPD and XRCC3 gene polymorphisms with oral squamous cell carcinoma in a Northeastern Brazilian population: A pilot study	The presence of the XRCC3-Met allele seems to contribute to the development of OSCC, metastases and more advanced stages in these lesions.
8	Kayani *et al*., 2014	Association of RAD 51 135 G/C, 172 G/T and XRCC3 Thr241Met Gene Polymorphisms with Increased Risk of Head and Neck Cancer	RAD51 (135G/C, 172 G/T) and XRCC3 (Thr241Met) polymorphisms may be effective biomarkers for genetic susceptibility to HNC. Larger studies are needed to confirm our findings and identify the underlying mechanisms
9	Khilfi *et al.,* 2013	DNA repair gene polymorphisms and risk of head and neck cancer in the Tunisian population	Our study suggests that only the *XRCC1*Arg399Glnpolymorphism was associated with the risk of HNC in the Tunisian population Furthermore, the risk of HNC was associated with *XRCC1* Arg399Gln polymorphism stratified by occupational exposure status. However, no statistically significant association was observed between the risk of developing HNC and the ERCC2 Lys751Gln and ERCC3 A>G
10	Alsbeih *et al*., 2013	SNPs in genes implicated in radiation response are associated with radiotoxicity and evoke roles as predictive and prognostic biomarkers	This study suggests that radiation toxicity in radiotherapy patients are associated with certain SNPs, in genes including *HDM2* promoter studied for the 1st time. These results support the use of SNPs as genetic predictive markers for clinical radio sensitivity and evoke a prognostic role for length of patients' follow-up after radiotherapy.

11	Al-Hadyan *et al.,* 2012	Involvement of Single-Nucleotide Polymorphisms in Predisposition to Head and Neck Cancer in Saudi Arabia	The p21 C31A, Ku80 A2790G, and MDM2 T309G genetic polymorphic variations are associated with the occurrence of head and neck cancer in the Saudi population. These SNPs could be used as genetic biomarkers to screen individuals at high cancer risk.
12	Wang *et al.,* 2012	Association between XRCC1 and XRCC3 gene polymorphisms and risk of thyroid cancer	The current study suggests that XRCC1 194 (Arg>Trp) and XRCC3 241 (Thr>Met) polymorphisms may be associated with TC risk in a Chinese population, especially in smokers and drinkers.
13	Kostrzewska-Poczekaj *et al.,* 2012	Polymorphisms of DNA repair genes and risk of squamous cell Carcinoma of the head and neck in young adults.	In head and neck cancer of young adult patients only homozygous AA variant in XRCC1 gene and heterozygous CT variant in XRCC3 gene were statistically significant. In conclusion, we could speculate on some contribution of XRCC1 and XRCC3 as risk factors for head and neck cancer development, but first of all environmental exposure, tobacco smoking and genes–environment interactions should be taken into account while estimating this risk.
14	Dos Reis *et al*., 2012	Allelic variants of XRCC1 and XRCC3 repair genes and susceptibility of oral cancer in Brazilian patients	Allelic variants of XRCC1 and XRCC3 are not suitable markers for susceptibility to carcinomas of the oral cavity and are also not related to the later stages of such tumors.
15	Mahjabeen *et al.,* 2012	Genetic Variations in *XRCC1* Gene in Sporadic Head and Neck Cancer (HNC) Patients	In this study they report the significant presence of Pro206Pro, Gln632Gln, Tyr576Asn and Arg399Gln genetic changes in head and neck cancer patients. The data suggests that a combination of silent mutations and missense mutations, or another mutation linked to it in the same gene or gene in vicinity, could conceivably play a role in the

			process of developing HNC cancer in a Pakistani population.
16	García-Quispes *et al*., 2011	Association studies of OGG1, XRCC1, XRCC2 and XRCC3 polymorphisms with differentiated thyroid cancer	From all the studied polymorphism, only a positive association was obtained for *XRCC1* (Arg280His). No Associations were observed for the other polymorphisms. No effects of the histopathological types of tumor were found when the thyroid cancer patients were stratified according to the type of tumor.
17	Azad *et al*., 2011	Validation of Genetic Sequence Variants as Prognostic Factors in Early-Stage Head and Neck Squamous Cell Cancer Survival	Two OS associations were statistically significant for each variant allele when compared with the wild-type: CTLA4:A49G (rs231775) and XRCC1: Arg339Gln (rs25487). Both of these sequence variants had significant results in the opposite direction as prior published literature. Two DFS associations were of borderline significance in the same direction as prior literature: ERCC2:Lys751Gln and TP53: Arg72Pro comparing number of variant alleles with reference of zero variants.
18	Fard-Esfahani *et al.,* 2011	Association of Arg194Trp, Arg280His and Arg399Gln Polymorphisms in X-ray Repair Cross-Complementing Group 1 Gene and Risk of Differentiated Thyroid Carcinoma in Iran	Genotyping of codons 194, 280 and 399 in *XRCC1* gene may use in risk assessment of DTC.
19	Ryu *et al*., 2011	*XRCC1* Polymorphisms and Risk of Papillary Thyroid Carcinoma in a Korean Sample	The XRCC1 Arg194Trp Arg/Trp genotype could be used as a useful molecular biomarker to predict genetic susceptibility for papillary thyroid carcinoma in Koreans.
20	Laantri *et al.,* 2011	XRCC1 and hOGG1 genes and risk of nasopharyngeal carcinoma in North African countries	Genetic variation at the XRCC1 and hOGG1 genes likely plays a negligible role in genetic susceptibility to NPC in the North African population, although we cannot rule out associations with NPC of other alleles at these loci.

21	Sliwinski *et al*., 2010	Polymorphisms of the XRCC3 C722T and the RAD51 G135C genes and the risk of head and neck cancer in a Polish population	The C722 T and the G135C polymorphisms of the XRCC3 and the RAD51 genes might be associated with HNSCC. Finally they suggest that these polymorphisms might be used as predictive factor of precancerous lesion for head and neck cancer in a Polish population.
22	Kowalski *et al*., 2009	Genetic polymorphisms in DNA base excision repair gene *XRCC1* and the risk of squamous cell carcinoma of the head and neck	They identified the combined Arg194Trp-Arg399Arg genotype of base excision repair gene *XRCC1* that was associated with HNSCC and may have an impact on identification of a high-risk cancer population.
23	Csejtei *et al*., 2009	Association between *XRCC1* Polymorphisms and Head and Neck Cancer in a Hungarian Population	No significant difference was found between patients and controls in respect of investigated polymorphisms. A significant difference was found between the patients with different XRCC1 194 polymorph status in clinical stage SIII. The survival proportion of patients with the Arg194Arg genotype was significantly lower than of those with the Arg194Trp genotype.

TABLE 2 Studies conducted on XRCC1 and XRCC3 genes (National)

S.NO.	Author and year	Title of paper	Outcomes of paper
1	Borkotoky *et al*., 2019	Alterations in XRCC1 gene profile in pathogenesis of oral cavity cancer: A population – based study from Kamrup Urban District of Assam, India	The presence of XRCC1 codon 399polymorphism was found to be associated with significantly increased risk of oral cavity carcinoma.
2	Swarup Nanda S. *et al*., 2018	Evaluation of XRCC-1 Gene Polymorphism as a Biomarker in Head and Neck Cancer Patients Undergoing Chemoradiotherapy.	Polymorphic variant XRCC-1 HNSCC patients treated with CCRT have significantly increased acute radiation morbidities and may have a trend towards better PFS in comparison to wild variant.
3	Narayana *et al*., 2018	Single Nucleotide Polymorphisms of XRCC1 and the Risk of Head and	The present study confirms the principle role of cigarette smoking and alcohol consumption in HNC. This is significant and positive

		Neck Cancer	interaction between these two factors. The frequency of heterozygous genotype (A/G) of *XRCC1* gene in both cases and controls showed no significant difference and hence, there was no association between them.
4	Raju *et al*., 2017	Polymorphic Variation in Double Strand Break Repair Gene in Indian Population: A Comparative Approach with Worldwide Ethnic Group Variations	The frequency in these DNA repair genes exhibit distinctive pattern in India that could be attributed to ethnicity variation. This could assist in high-risk screening of humans exposed to environmental carcinogens and cancer predisposition in different ethnic groups.
5	Kailas *et al*., 2016	Role of genetic polymorphisms in DNA repair genes (xrcc1, xrcc2, xrcc3, xrcc4, xrcc5, xrcc6, xrcc7) in head and neck cancer susceptibility in rural Indian population: a hospital-based case -control study from south western Maharashtra.	This study indicates that variant types of XRCC1, XRCC4, XRCCC5 and XRCC7 genes play a role in modifying genetic susceptibility of individual to head and neck cancer.
6	Kumar *et al*., 2012	Associated risk of XRCC1 and XPD cross talk and life style factors in progression of head and neck cancer in north Indian population	The high degree of gene–gene interaction involving DNA repair genes of NER and BER pathways, namely XRCC1 and XPD. This study amply demonstrates positive association of XPD Arg751Gln polymorphism with an increased risk of SCCHN. Further, XRCC1 Arg280His variant though dormant individually, may also contribute to the development of cancer in combination with XPD Arg751Gln.

Objective of the study

The objective of this study is:

- To investigate the role of polymorphisms in double strand break repair genes XRCC1 and XRCC3 in head and neck cancer incidences
- To correlate the polymorphism of XRCC1 and XRCC3 with efficacy of radiation therapy

METHODOLOGY

Study subjects

The study must be hospital based and it shall be case control study conducted in Rajasthan from India. Study participants must include the patients which are newly diagnosed with head & neck cancer and normal person of same age and sex matched selected as controls. We have to collect raw data about cases and controls by interviewing them personally about their occupational history, dietary habits, smoking and drinking status, demographic factors (e.g. age sex and ethnicity) and known risk factors, and individual family history of diseases, including HNC cancer.

NAME: **DATE OF COLLECTION:** **DATE OF BIRTH:** **ADRESS:** **CAST AND RELIGION:**		
S.NO.	**Variable**	**Tick accordingly**
1	**Age**	0-25 ( ) 25-50 () 50-75( ) 75 and above( )
		Specific age ( )
2	Sex	Male ( ) Female ( ) other ( )
3	Tobacco chewing/smoking Status	Smokers ( ) Nonsmokers ( ) Tobacco (branded/any other form)
		If yes time period -
4	Alcohol status	Drinkers( ) Non-Drinkers ( )
		If yes time period -
5	Diet	Vegetarians( ) Non vegetarians( )
6	Education	High School( ) High School graduate ( )
		College /Graduate( ) Post graduate ( )
7	Economic status	Middle ( ) Poor ( )Other( )
8	Family history of Cancer	Yes ( ) No ( ) If yes from which side
		Paternal ( ) Maternal ( )
		type of cancer -
9	Marital status	Yes ( ) No ( ) If yes how many years ( )

10	Children	
10	Disease categories	
	Stage	High (III+IV) ( ) Low(I+II) ()
	Grade	>1 ( ) 1 ( )
	T-status	T3+T4 ( ) T1+T2 ( )
	Lymph node	N2+N3 ( ) N0+N1 ( )
	Metastasis	M1 ( ) M0 ( )
	Hemoglobin level	High ( ) Low ( )
11	Site of cancer	Alveolus ( ) Tongue ( ) Buccal mucosa( )
		Submandibular lymph node ( )Pharynx ( )
		Larynx ( ) Supraglottic region ( )
12	Treatment method	
13	Treatment Outcome	

Genomic DNA isolation from whole blood

Genomic DNA extraction should be carried out from the blood sample (Miller *et al*., 1988). After this, quantitative and qualitative analysis of genomic DNA shall be carried out.

Genotyping assays

Genotyping of XRCC genes (XRCC1 and XRCC3) is to be performed by **PCR-RFLP method** with appropriate primer sets (Narayana *et al*., 2018). The primers will be designed to amplify the regions of DNA that contain polymorphic sites of interest: XRCC1 Arg399Gln in the exon 10 (G>A), XRCC3 Thr241Met in the exon 7.

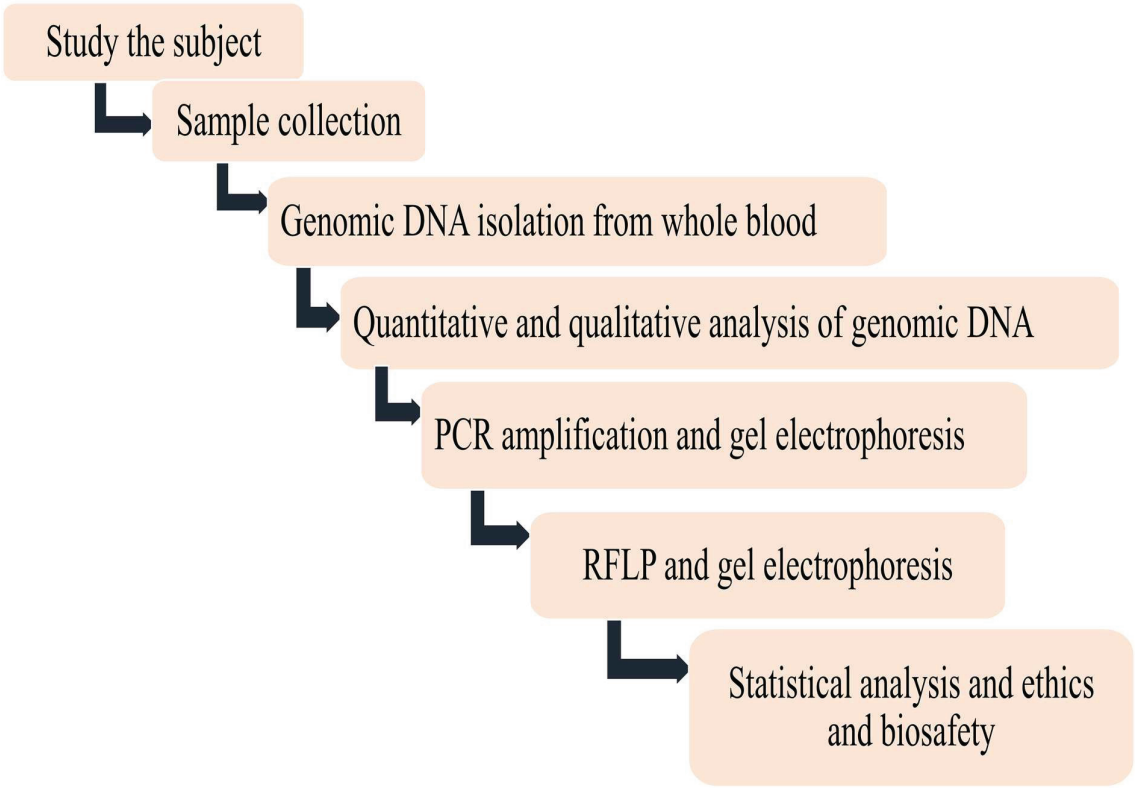
Statistical analysis

All statistical analysis must be implemented by using IBM SPSS statistics (version 20.0) by IBM Corp US.

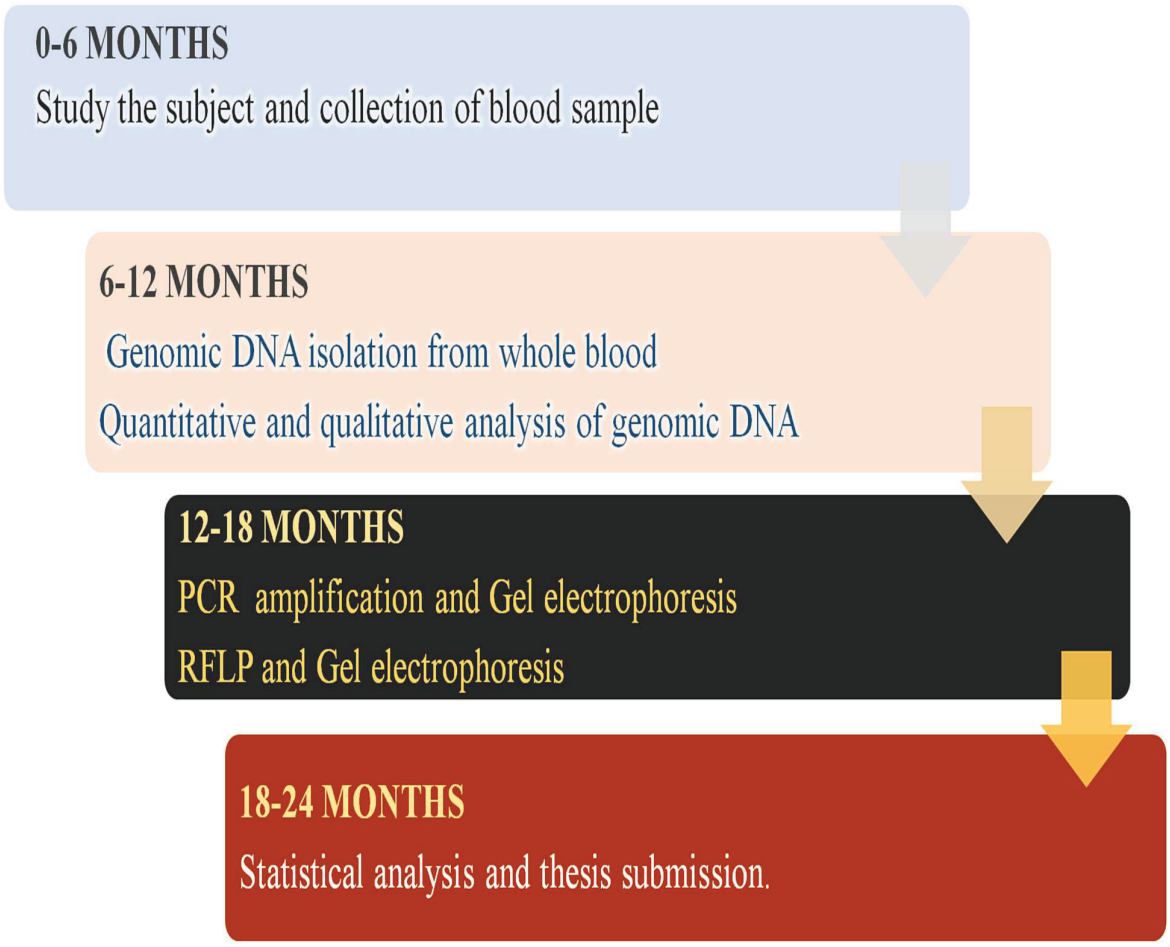
Ethics and bio safety

The study protocol must be approved by well recognized medical community.

FLOW CHART OF METHODOLOGY


Study the subject
Sample collection
Genomic DNA isolation from whole blood
Quantitative and qualitative analysis of genomic DNA
PCR amplification and gel electrophoresis
RFLP and gel electrophoresis
Statistical analysis and ethics and biosafety

PLAN OF WORK


0-6 MONTHS
Study the subject and collection of blood sample
6-12 MONTHS
Genomic DNA isolation from whole blood
Quantitative and qualitative analysis of genomic DNA
12-18 MONTHS
PCR amplification and Gel electrophoresis
RFLP and Gel electrophoresis
18-24 MONTHS
Statistical analysis and thesis submission.

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